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Effect of Irradiation on Incidence of Post-Transplant Lymphoproliferative Disorder after Hematopoietic Cell Transplantation in Miniature Swine

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Post-transplant lymphoproliferative disease (PTLD) is a major complication of clinical organ and cell transplantation. Conditioning and immunosuppressive regimens that significantly impair T cell immunity, including depleting antibodies and calcineurin inhibitors, increase the risk of PTLD after transplantation. Swine PTLD has been shown to closely resemble human PTLD in morphology, histology, and viral-driven reactivation of B cells. Previously, we reported high incidences of PTLD after hematopoietic cell transplantation (HCT) in miniature swine recipients conditioned with thymic irradiation (TI) in addition to T cell depletion and cyclosporine A monotherapy after transplantation. Replacement of TI with 100 cGy of total body irradiation resulted in similar numbers of B cells early post-transplantation, greater numbers of T cells at day 0, and markedly decreased incidence of PTLD, suggesting that a threshold number of T cells may be necessary to prevent subsequent B cell proliferation and development of overt PTLD. Results from this large cohort of animals provide insight into the important effect of irradiation and T cell immunity on the incidence of PTLD after HCT and reinforce the pig model as a valuable tool for the study of PTLD and HCT.

INTRODUCTION

Post-transplant lymphoproliferative disease (PTLD) is a potentially lethal complication of clinical organ and cell transplantation, as a consequence of prolonged immunosuppression [1]. In humans, PTLD is characterized by an abnormal B cell proliferation related to Epstein-Barr virus primary infection or reactivation. Conditioning regimens that diminish T cell immunity, such as thymoglobulin mediated T cell depletion or long-term calcineurin inhibition, increase the risk of PTLD after transplantation. This risk results from the loss of antiviral function of $CD8^+$ cytotoxic T cells, which are primarily responsible for suppressing Epstein-Barr virus–associated infections [2]. The variability of the human patient population, both clinically and pathologically, complicates the ability to study PTLD. Murine models of PTLD involving immunodeficient mice injected with human PTLD lines and mice infected with murine gamma herpesvirus [3,4] are often unreliable and do not model human disease accurately. Therefore, large animal preclinical models that mimic human PTLD provide an opportunity to identify risk factors and investigate therapeutic approaches to mitigate this frequently lethal condition.

We have previously reported a high incidence (>33%) of PTLD in swine after hematopoietic cell transplantation (HCT) that closely resembles human PTLD both morphologically and histologically [5,6]. In this model recipients were conditioned with T cell depletion using a porcine CD3 immunotoxin and cyclosporine A (CyA) monotherapy with or without thymic irradiation (TI; 700 to 1000 cGy). Risk factors for the development of PTLD in this model, which are similar to those in humans, were also described, including the effects of peripheral blood chimerism levels, T cell depletion, TI, immunosuppression levels, and MHC disparity [6]. We also identified a novel porcine lymphotropic herpesvirus-1 (PLHV-1) associated with PTLD in swine, paralleling human infection with Epstein-Barr virus [7]. These findings further support the pig as a reliable preclinical large animal model of PTLD.

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In an attempt to reduce the incidence of PTLD in this model, we replaced high-dose TI as part of the preparative regimen with low-dose 100 cGy of total body irradiation (TBI). In this study, we report on the effect of irradiation (TBI versus TI) on the incidence of PTLD in our miniature swine model and also demonstrate that serum lactate dehydrogenase (LDH) levels serve as a supportive marker for the diagnosis of PTLD in swine.

METHODS

Animals

This is a retrospective study of all animals conditioned for HCT at the Transplantation Biology Research Center at Massachusetts General Hospital using a porcine CD3 immunotoxin for recipient T cell depletion and a short course of CyA since 1997. All animals that developed PTLD were diagnosed between 21 and 45 days post-HCT. Therefore, only animals who survived at least 45 days post-HCT were selected for this analysis, unless early death was due to PTLD. Animals were selected from our herd of Massachusetts General Hospital partially inbred MHC-defined miniature swine, which have been previously described [8]. Transplant donors ranged from 4 to 6 months in age and recipient animals from 8 to 12 weeks. All experiments were approved and performed in compliance with the Institutional Animal Care and Use Committee.

Irradiation

When administered, animals received either 100 cGy of TBI or 1000 cGy of TI as previously described [9]. Because only 4 animals received 700 cGy TI in 1997 before the protocol was changed to 1000 cGy TI, we decided to exclude these 4 animals from the PTLD analysis in this study. Animals were sedated, and a cobalt irradiator was used for all regimens. The dose was calculated dependent on the source decay charts.

T Cell Depletion and CyA

Animals receiving no irradiation or TI were administered .05 mg/kg CD3 immunotoxin, pCD3-CRM9 [10], on day −2 for T cell depletion. Animals receiving TBI were either administered .05 mg/kg CD3 immunotoxin, pCD3-CRM9, on day −2 (n = 13) or .05 mg/kg recombinant CD3-immuntoxin (pCD3-rIT) [11] twice daily 8 hours apart beginning on day −4 until day −1 (n = 27). CyA was administered orally through a gastrostomy tube twice daily with target levels of 400 to 800 ng/mL in all animals commencing on day −1 and concluding on either day 30 or 60 for those animals receiving TI or day 30 followed by a 2-week taper for those animals receiving TBI.

Hematopoietic Cell Transplantation

HCT was performed as previously described [12]. Briefly, transplant donors were mobilized once daily with porcine IL-3 (100 μg/kg/day) and porcine stem cell factor (SCF; 100 μg/kg/day) or with human granulocyte colony-stimulating factor (G-CSF; 10 μg/kg/day) beginning on day −5. Peripheral blood mononuclear cells (PBMCs) were harvested by leukapheresis beginning on day 0. Cytokine injections were continued, and donor animals were leukapheresed daily for up to 3 days until the target cell dose of 1 to 15×10^9 cells/kg of recipient body weight was collected. After each leukapheresis collection, fresh cells were infused intravenously into recipient animals over 15 to 20 minutes.

PTLD Diagnosis

A diagnosis of PTLD was made based on a combination of flow cytometric confirmation of B cell lymphoproliferation and physical signs and symptoms, including loss of appetite, lethargy, and lymphadenopathy. In the case of death, confirmation of PTLD diagnosis was made by histologic analysis. All recipients that developed PTLD were diagnosed between 21 and 45 days post-HCT.

Flow Cytometry

Flow cytometry was performed on a weekly basis to monitor myeloid and B cell proliferation, chimerism levels, and percent and absolute T cell counts in the peripheral blood. Methods were previously described [5,6]. Briefly, chimerism levels were assessed by the use of pig allelic antigen, a nonspecific cell marker present on donor cells but not on host cells. Donor and hosts were specifically screened before transplant, and only pig allelic antigen–positive donors and pig allelic antigen–negative hosts were selected. B cell lymphoproliferation and absolute B cell numbers were monitored by assessing the $CD3^-/CD16^-$ double-negative population as previously described [5,6].

Graft-versus-Host Disease Assessment

Graft-versus-host disease (GHVD) was assessed daily by physical exam in all animals. Total bilirubin and liver enzymes (alanine and aspartate aminotransferases and alkaline phosphatase) were assessed biweekly (or more often if medically indicated). Any rash was documented, and skin was biopsied and assessed via histopathology if deemed appropriate. Gastrointestinal signs of GVHD were assessed clinically by fecal volume (animals housed in metabolic cages) and consistency (watery, mucoidal, mucohemorrhagic, etc). Additionally, rectal biopsies were taken if clinically indicated and compared with pretransplant samples. Twice weekly, donor and host T cells were assessed via flow cytometry. A Seattle scoring system was developed in swine and used to standardize GVHD assessment (developed in conjunction with Dr. Thomas R. Spitzer- director of the Massachusetts General Hospital unit). A description of this GVHD swine scoring system and the characterization of the miniature swine GVHD is forthcoming (Duran-Struck et al, unpublished data).

LDH ELISA

PBMCs were isolated from heparinized blood by Ficoll gradient centrifugation and cultured for 18 hours with or without phytohemagglutinin (M Form; Invitrogen, Carlsbad, CA, USA). Cells were plated at 1×10^3 to 5×10^4 cells per well, and total LDH was determined using the Lactic Dehydrogenase based In Vitro Toxicology Assay Kit (Sigma Aldrich, St. Louis, MO, USA). This assay has been previously described [13].

Statistical Analysis

A 1-sample *t*-test was used to assess whether the leukocyte counts were higher among the PTLD animals than the mean of the normal range under the assumption of unknown variance. A 2-sample *t*-test was used to compare the distribution of LDH levels between the PTLD animals versus healthy controls under the assumption of unequal variances. The *P* values were based on a 2-sided hypothesis test and computed using Stata 7.0 (Stata Corp, College Station, TX). Survival curves were plotted using Kaplan-Meier estimates using a log rank (Mantel-Cox) test. $P < .05$ was considered statistically significant.

RESULTS

Effect of Irradiation on Incidence of PTLD after HCT

Beginning in 1997, 29 miniature swine received SCF/IL3 mobilized HCTs after conditioning with 1000 cGy TI, T cell depletion with pCD3-CRM9, and a short course of CyA (30 to 60 days), with a PTLD incidence rate of 34.4% (10/29) (Figure 1). In an attempt to reduce post-transplant complications including PTLD in this model of HCT, TI was removed from the protocol, which resulted in a decreased incidence of

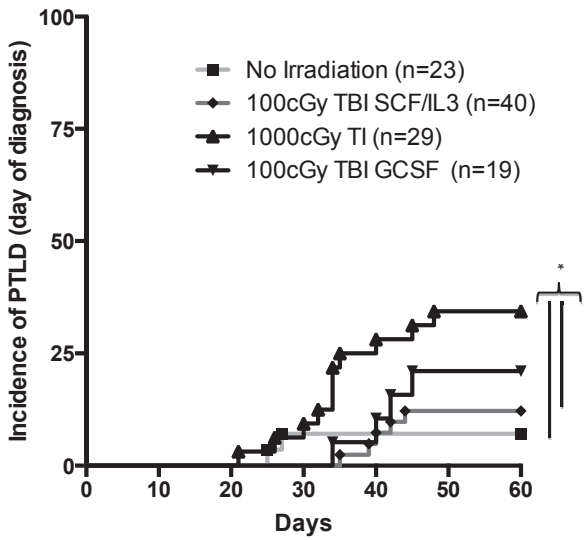

Figure 1. PTLD incidence. Incidence of PTLD in animals that received a conditioning regimen with SCF/IL3 + no irradiation (■), SCF/IL3 + TBI (◆), SCF/IL3 + TI (▲), or G-CSF + TBI (▼). *$P < .05$.

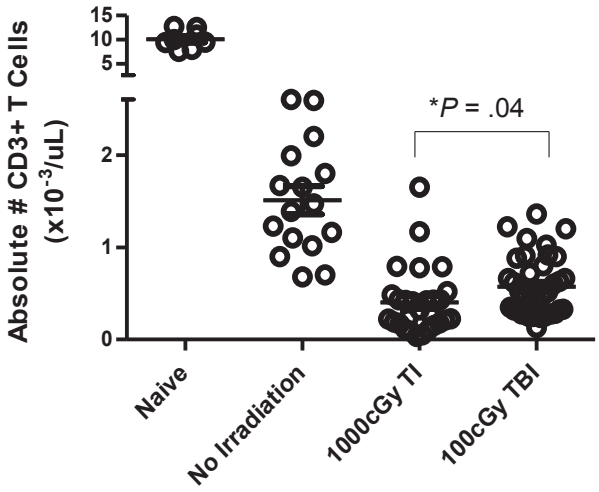

Figure 2. T cell depletion. Level of T cell depletion in animals on day 0 after receiving a conditioning regimen with CD3 immunotoxin and either no irradiation (n = 16), 1000 cGy TI (n = 28), or 100 cGy TBI (n = 37). Absolute $CD3^+$ T cells from naive swine are provided for comparison.

PTLD of 4.3% in 23 animals (1/23) (Figure 1). However, this very mild preparatory regimen consisting of only CD3-immunotoxin and CyA monotherapy without TI resulted in reduced T cell depletion (Figure 2), and many animals failed to engraft long term [14]. As a result, 100 cGy of TBI was added to the conditioning protocol with the intention of providing additional immunosuppression and/or bone marrow space to enhance engraftment, while also depleting radiosensitive B cells. In 40 subsequent HCTs of SCF/IL3 mobilized cells, 12.5% (5/40) developed PTLD (Figure 1). Thirty-seven animals were transplanted across a single-haplotype mismatch and 3 across a 2-haplotype mismatch with PTLD incidences of 10.8% (4/37) and 33.3% (1/3), respectively (Table 1).

No Effect of Mobilization and T Cell Depletion Strategies on PTLD

Using a different mobilization strategy, 19 animals underwent the same conditioning regimen with T cell depletion and 100 cGy TBI but received G-CSF mobilized cells. All 19 were transplanted across a single-haplotype mismatch and had a PTLD incidence of 21% (4/19) (Table 1 and Figure 1). Among the animals in the TBI groups, there was no significant difference ($P = .38$) in PTLD incidence between recipients of G-CSF versus SCF/IL3 mobilized cells (Figure 1). In total, 59 animals underwent reduced-intensity conditioning with 100 cGy TBI and were transplanted with either SCF/IL3 (n = 40) (Figure 1) or G-CSF (n = 19) (Table 1) mobilized cells, with an overall PTLD incidence rate of 15.2% (9/59).

As described in Methods, 2 different CD3 immunotoxins were used during the course of this study. pCD3-CRM9 was substituted for pCD3-rIT for safety reasons unrelated to this study and the development of PTLD. Animals receiving no irradiation (n = 23) and TI (n = 29) received T cell depletion with pCD3-CRM9 on day −2. Among 40 animals receiving SCF/IL3 + TBI, 13 animals received T cell depletion with pCD3-CRM9 on day −2 and 27 with pCD3-rIT twice daily from days −4 to −1 (Supplementary Figure 1B,C). Of the 5 animals in the SCF/IL3 + TBI group who developed PTLD post-transplant, 4 of 27 (14.8%) had been conditioned with pCD3-rIT and 1 of 13 (7.8%) with pCD3-CRM9. All 19 animals in the G-CSF + TBI group received pCD3-CRM9, with 4 (21%) developing PTLD. Although the overall number of animals is limited, our results support that pCD3-CRM9 versus pCD3-rIT had no statistically significant effects on PTLD development (Supplementary Figure 1B,C).

The majority of PTLD in the TI group was of host type lymphoproliferation (9/10). Of the 9 total animals that developed PTLD in the TBI group, data on the origin of PTLD was only available in 3 animals, of which 2 were host origin and 1 was donor origin (Supplementary Figure 2).

Length of CyA Treatment and PTLD

We compared the incidence of PTLD among the different groups based on the length of CyA treatment. Among the TI group, 12 of 29 animals received a 30-day course of CyA and the remaining 17 were scheduled to receive a 60-day course. Three of 12 animals (25%) that received CyA for 30 days developed PTLD. All were diagnosed early after the discontinuation of immunosuppression (days 30, 32, and 35). Of the remaining 17 animals scheduled for a 60-day CyA course, 7 (41%) developed PTLD (CyA was subsequently decreased or discontinued in these animals). All of them developed PTLD early post-HCT (days 21, 26, 34, 34, 40, 45, and 48). Among the 40 animals in the SCF/IL3 + TBI group, all received a 45-day course of CyA, which included continuous dosing through day 30 followed by a 2-week taper to day 45. Five of 40 developed PTLD (12.5%). Similarly, all 19 animals in the G-CSF + TBI group also received a 45-day course of CyA, with 4 (21%) developing PTLD. We did not find a correlation between length of CyA and development of PTLD post-transplant (Supplementary Figure 1A).

MHC Mismatch and PTLD

Within the TI group, where the incidence of PTLD was greatest, we did not observe any significant difference in incidence among animals that were transplanted across single-haplotype MHC barriers (5/14; 35.7%) compared with those transplanted across 2-haplotype MHC barriers (5/14; 35.7%) (Table 1). Within the TBI group, animals transplanted across a single-haplotype MHC barrier appeared to have a lower incidence of PTLD (4/37 or 10.8%) than those transplanted across 2 MHC haplotype barriers (1/3 or 33.3%). However, the total number of animals developing PTLD (n = 5) and the total number of animals transplanted across

Table 1
MHC Disparity and PTLD Incidence

	No Irradiation SCF/IL-3 (No. Developing PTLD; PTLD Incidence)	TI SCF/IL-3 (No. Developing PTLD; PTLD Incidence)	TBI SCF/IL-3 (No. Developing PTLD; PTLD Incidence)	TBI G-CSF (No. Developing PTLD; PTLD Incidence)
No mismatch	2 (0; 0%)	1 (0; 0%)	0	0
Single-haplotype mismatch	16 (1; 6.2%)	14 (5; 35.6%)	37 (4; 10.8%)	19 (4; 21.1%)
Two-haplotype mismatch	5 (0; 0%)	14 (5; 35.6%)	3 (1; 33.3%)	0
Total	23 (1; 4.3%)	29 (10; 34.4%)	40 (5; 12.5%)	19 (4; 21.1%)

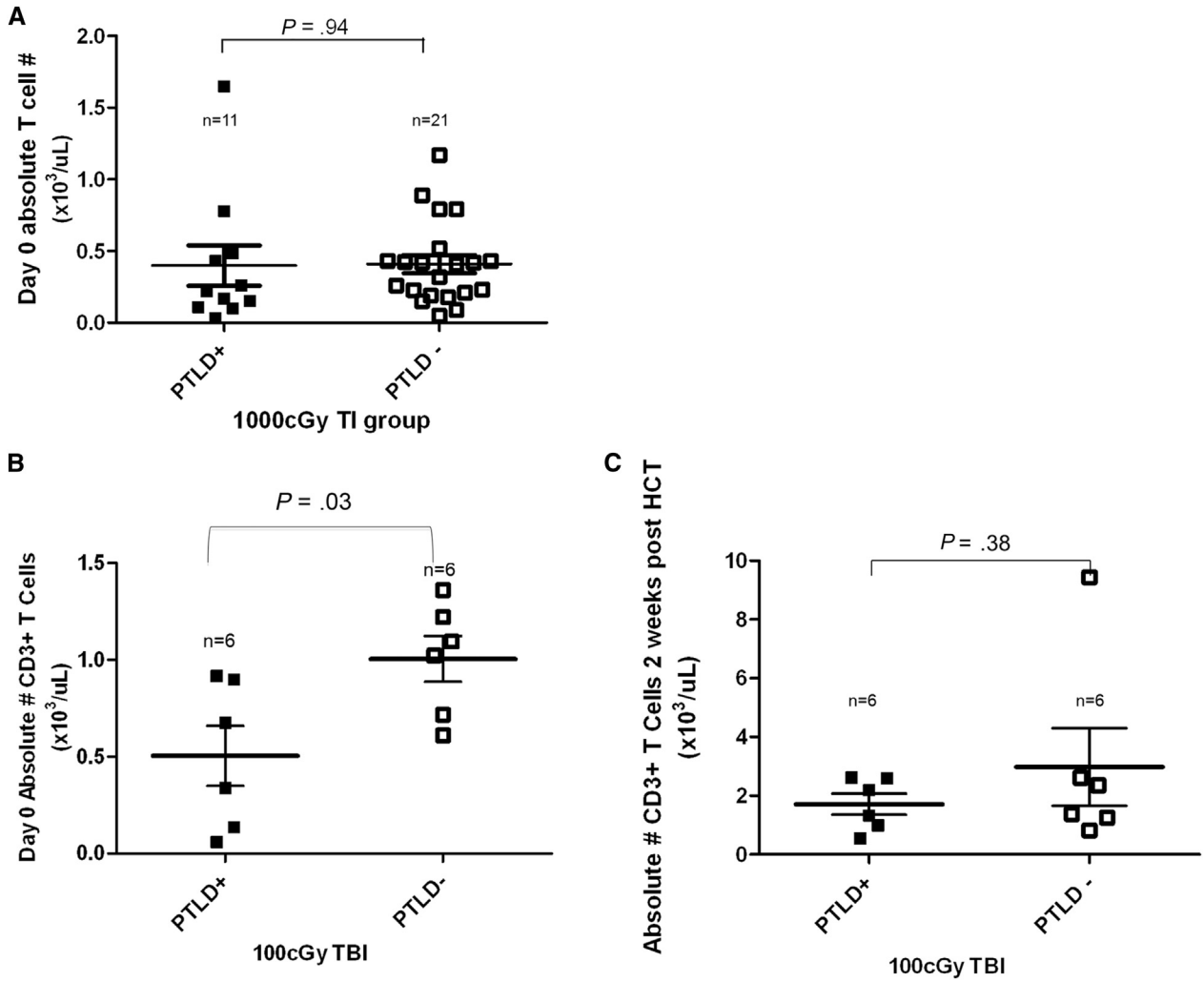

Figure 3. Comparison between absolute T cell numbers in animals with and without PTLD after receiving different conditioning regimens. (A) Post-HCT day 0 absolute T cell counts in animals treated with TI. (B) Absolute T cell counts on post-HCT day 0 after conditioning with TBI. (C) Absolute T cell counts 2 weeks post-HCT in animals that received SCF/IL3 with TBI.

2-haplotype MHC barriers (n = 3) among the TBI group are too small to make any conclusions regarding this point (Table 1).

Effect of Lymphocyte Depletion and Recovery on Incidence of PTLD

Absolute counts of T cells on day 0 in animals conditioned with TI, 0.45 × 10^3/uL ± 0.35 (n = 28), were found to be significantly lower ($P = .04$) than those conditioned with TBI, 0.57 × 10^3/uL ± 0.33 (n = 37) (Figure 2). However, there was no significant difference in absolute T cell numbers at day 0 between those animals developing PTLD, 0.39 × 10^3/uL ± 0.47 (n = 11), and those that did not, 0.41 × 10^3/uL ± 0.28 (n = 21), within the TI group (Figure 3A). In contrast, the day 0 T cell counts were found to be significantly lower ($P = .03$) in the animals that developed PTLD, 0.5 × 10^3/uL ± 0.38 (n = 6), than those that did not, −1.0 × 10^3/uL ± 0.29 (n = 6), within the group that received TBI (Figure 3B). Conversely, absolute T cell recovery measured at 2 weeks in the same group revealed no significant differences between animals that developed PTLD from those that did not (Figure 3C). A comparison of absolute B cell numbers at 1 and 2 weeks post-HCT between animals in the TI versus TBI groups showed no significant differences (data not shown). A limitation of this analysis, however, is that B cell data were available from only 4 animals in the TI group at these early time points.

Serum LDH is a Reliable Marker in the Diagnosis of PTLD in Miniature Swine

Retrospective analysis of serum LDH levels was performed in 17 HCT recipients, regardless of conditioning regimen. Mean LDH level at the time of PTLD diagnosis in 9 animals that developed PTLD (6034 U/L) was significantly higher than in 8 HCT recipients that did not develop PTLD (1392 U/L) when analyzed at similar time points (Figure 4A). Furthermore, analysis of serum taken before any clinical presentation of lymphadenopathy showed an increase in LDH level at the time of WBC increase in animals that developed PTLD (Figure 4B). Previously, we reported the establishment of porcine PTLD tumor cell lines [15]. We analyzed 5 porcine PTLD tumor cell lines for production of LDH in vitro and compared with LDH production from normal PBMCs in vitro. PTLD cell lines secreted greater amounts of LDH than PBMCs as measured by ELISA (0.2 optical density versus mean 0.4 optical density) (Figure 4C).

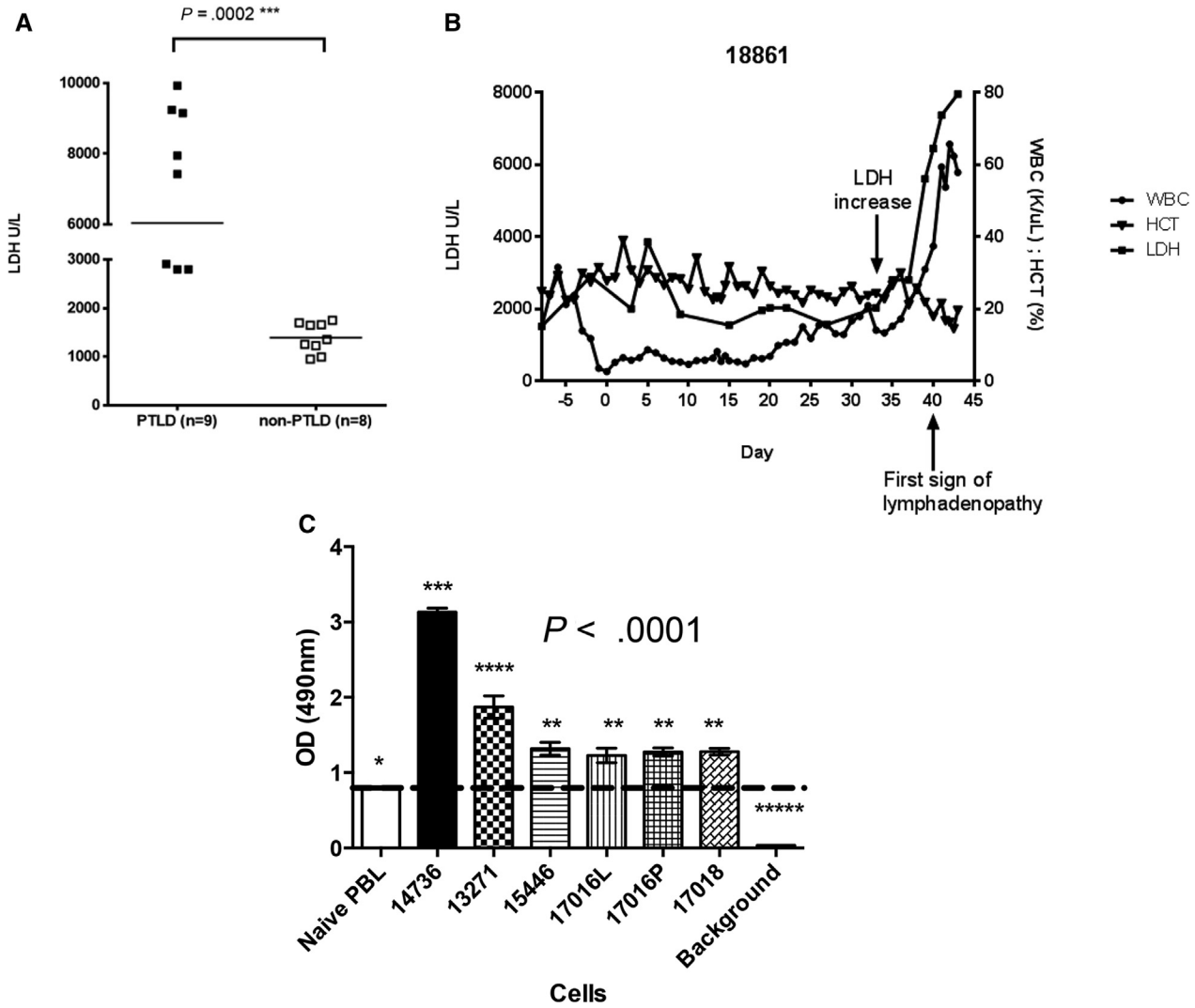

Figure 4. LDH. (A) Representative serum LDH levels (U/L) at the time of PTLD diagnosis (6034 U/L) compared with serum LDH levels in animals without PTLD (1392 U/L) ($P = .0002$). (B) Representative data of serum LDH levels over time from pre-HCT to clinical lymphadenopathy in an animal (18861) developing PTLD. (C) LDH levels detected by ELISA in 5 previously established porcine PTLD tumor cell lines (14736, 13271, 15446, 17016L [lymph node origin], 17016P [peritoneal origin], 17018) compared with LDH levels from normal peripheral blood leukocytes. Statistical significance with a $P < .0001$ between groups marked with different number of asterisks (*). Groups that have the same number (*) are not statistically significant between each other.

DISCUSSION

We previously reported that a novel HCT protocol consisting of 700 to 1000 cGy TI, T cell depletion with pCD3-CRM9, and CyA monotherapy resulted in high PTLD incidence rates (>33%) [5,6]. Removal of TI reduced the incidence of PTLD, but, similarly to the TI group, poor long-term engraftment outcomes were observed. Subsequently, 100 cGy TBI was introduced to the preparative regimen in an attempt to improve donor cell engraftment while simultaneously hypothesizing that the effect of TBI on peripheral B cells would minimize the risk of developing PTLD. This change significantly reduced the incidence of PTLD while improving long-term engraftment outcomes without increasing the incidence of GVHD in our model 12% (Duran-Struuck et al., unpublished data). One hypothesis for improved donor cell engraftment upon introduction of TBI is the increased "space" in the marrow niches provided by TBI mediated ablation of hematopoietic cells [16,17].

In comparison with solid organ transplantation, reactivation (or de novo infection) of PLHV-1 and development of PTLD after allogeneic HCT generally appears early in the post-transplant period when T cell responses are blunted [18]. Thus, limiting the period of impaired T cell immunity after transplantation is a critical component in preventing PTLD. Using our reduced-intensity conditioning regimen, animals regain T cell immunity within 7 weeks after transplantation demonstrated by third-party cell mediated lympholysis reactions (data not shown). Furthermore, absolute T cell numbers are only transiently reduced and begin to recover within 10 to 14 days after transplantation (data not shown).

We initially hypothesized that the combination of greater levels of T cell depletion in the animals receiving 1000 cGy TI and the lack of any direct effect on B cells contributed to uncontrolled B cell proliferation and the development of PTLD—most likely stimulated via PLHV-1 viral infection or reactivation. Although not significant enough to make a conclusion because of the limited numbers of animals in the TI

group, a comparison of absolute B cell numbers at 1 and 2 weeks post-HCT from animals in each group (TI versus TBI) showed no differences. These preliminary data showing no apparent differences in B cell numbers contradict our initial hypothesis that the 100-cGy TBI regimen, and not the 1000-cGy TI regimen, would reduce host B cell numbers directly. Although peripheral blood B cell numbers were similar early post-transplantation, it is possible that animals receiving TBI may have had significantly lower numbers of B cells present in lymphoid tissues not sampled such as the lymph nodes or spleen. The dose of TBI used in this study is far less damaging to the thymus and thus may explain the difference in absolute T cell numbers between the 2 groups. Based on these data, it may be that a threshold number of T cells (and potentially sparing memory T cells) at day 0 are required to prevent subsequent B cell proliferation and development of overt PTLD. It is important to note that unlike other antibodies such as antithymocyte globulin, CD3 immunotoxin has significant depleting effects on thymic T cells, mitigating the need for TI in protocols already including CD3 immunotoxin [19].

We have previously reported on the incidence of PLHV-1 in our miniature swine herd, noting that its incidence increases with the age of the animal [6]. We previously estimated that 19% of young recipients (6 to 8 weeks of age) test positive for PHLV-1 and 55% of donors (4 to 6 months of age) test positive. Importantly, we found no correlation between pretransplant positive PLHV-1 status (either in donor or recipient) and post-transplant development of PTLD.

Cyclosporine monotherapy was used post-transplantation in all animals. Although the varying lengths of post-transplant immunosuppression courses could have played a role in PTLD incidence, all animals (who did not die of PTLD before day 30) received CyA immunosuppression for at least 30 days. This length of immunosuppression is sufficient to allow for the development of PTLD, as demonstrated by 4 of 11 who received TI and developed clinical PTLD before day 30. Moreover, 3 animals receiving 30-day courses of CyA were actually diagnosed on or after day 30 (days 30, 32, and 35).

As described, there was no significant difference in PTLD incidence among animals that were transplanted across single-haplotype MHC barriers (5/14; 35.7%) compared with those transplanted across 2 haplotype MHC barriers (5/14; 35.7%) in the TI group (Table 1). Within the TBI group, animals transplanted across a single haplotype MHC barrier appeared to have a lower incidence of PTLD (4/37 or 10.8%) than those transplanted across 2 MHC haplotype barriers (1/3 or 33.3%). However, as was stated, the distribution of the animals developing PTLD ($n = 5$) was limited by the total number of animals transplanted across 2 haplotype MHC barriers ($n = 4$). However, there was a noteworthy difference in the distribution of single- versus 2-haplotype mismatches among the TI and TBI groups. Fourteen of 29 animals (48.2%) in the TI group were transplanted across a 2-haplotype mismatch, whereas only 3 of 40 animals (7.5%) in the TBI group were transplanted across a 2-haplotype mismatch. We acknowledge that this variable may have had an influence on PTLD incidence, because MHC disparity may be another contributing factor. However, when comparing animals receiving the same mobilization regimen (SCF/IL3) and transplanted across a single-haplotype mismatch (14 animals in the TI group and 37 in the TBI group), animals receiving TBI had lower incidences of PTLD (10.8% versus 35.7%) than those in the TI group.

Similar to humans, serum LDH has already been shown to be elevated in swine chronic myeloid leukemias, but this correlation has not yet been demonstrated in swine PTLD B cell lymphomas until now. We have also previously demonstrated the similarity of porcine PTLD to human PTLD with respect to viral driven activation, morphologically and histologically [5,6]. Here we provide evidence that LDH can also serve as a supportive diagnostic marker for PTLD lymphomas. In our swine, the increase of LDH can be detected at least 1 to 2 days before the increase in WBC count and well before any clinical manifestation of lymphadenopathy. Therefore, similarly to human PTLD [20], rising serum LDH levels after transplantation can be an indicator of a lymphoproliferative process.

In summary, large animal models can be an invaluable tool for the study of PTLD after allogeneic HCT. This report provides insight into the effects of irradiation on the incidence of PTLD after HCT. Reduced-intensity conditioning regimens using 1000 cGy TI dramatically reduce T cell numbers, resulting in a significant incidence of PTLD. The addition of 100 cGy TBI appears to be protective of PTLD by maintaining a sufficient number of T cells in the host periphery, and preliminary data suggest this protective mechanism is not based on greater B cell depletion. Based on these outcomes, we report that the addition of 100 cGy TBI minimizes the risk of PTLD compared with 1000 cGy TI while allowing for acceptable levels of engraftment and a low incidence of GVHD. The use of pre-emptive low-dose total body irradiation warrants further inquiry in specific immunosuppressive regimens.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found at 10.1016/j.bbmt.2015.07.017.

REFERENCES

1. Shapiro RS, McClain K, Frizzera G, et al. Epstein-Barr virus associated B cell lymphoproliferative disorders following bone marrow transplantation. *Blood*. 1988;71:1234-1243.
2. Khatri VP, Baiocchi RA, Peng R, et al. Endogenous CD8+ T cell expansion during regression of monoclonal EBV-associated posttransplant lymphoproliferative disorder. *J Immunol*. 1999;163:500-506.

3. Randhawa PS, Pietrzak B, Nalesnik MA, et al. Subcutaneous implantation of human post-transplant lymphoproliferative disease lesions in SCID mice. *Hematol Oncol*. 1997;15:39-46.
4. Johannessen I, Bieleski L, Urquhart G, et al. Epstein-Barr virus, B cell lymphoproliferative disease, and SCID mice: modeling T cell immunotherapy in vivo. *J Med Virol*. 2011;83:1585-1596.
5. Huang CA, Fuchimoto Y, Gleit ZL, et al. Posttransplant lymphoproliferative disease in miniature swine after allogeneic hematopoietic cell transplantation: similarity to human PTLD and association with a porcine gammaherpesvirus. *Blood*. 2001;97:1467-1473.
6. Cho PS, Meuller NJ, Cameron AM, et al. Risk factors for the development of post-transplant lymphoproliferative disorder in a large animal model. *Am J Transplant*. 2004;4:1274-1282.
7. Goltz M, Ericsson T, Patience C, et al. Sequence analysis of the genome of porcine lymphotropic herpesvirus 1 and gene expression during posttransplant lymphoproliferative disease of pigs. *Virology*. 2002;294:383-393.
8. Sachs DH, Leight G, Cone J, et al. Transplantation in miniature swine. I. Fixation of the major histocompatibility complex. *Transplantation*. 1976;22:559-567.
9. Huang CA, Fuchimoto Y, Scheier-Dolberg R, et al. Stable mixed chimerism and tolerance using a nonmyeloablative preparative regimen in a large-animal model. *J Clin Invest*. 2000;105:173-181.
10. Huang CA, Yamada K, Murphy MC, et al. *In vivo* T cell depletion in miniature swine using the swine CD3 immunotoxin, pCD3-CRM9. *Transplantation*. 1999;68:855-860.
11. Wang Z, Duran-Struuck R, Crepeau R, et al. Development of a diphtheria toxin based antiporcine CD3 recombinant immunotoxin. *Bioconjug Chem*. 2011;10:2014-2020.
12. Cina RA, Wikiel KJ, Lee PW, et al. Stable multilineage chimerism without graft versus host disease following nonmyeloablative haploidentical hematopoietic cell transplantation. *Transplantation*. 2006;81:1677-1685.
13. Duran-Struuck R, Cho PS, Teague AG, et al. Myelogenous leukemia in adult inbred MHC-defined miniature swine: a model for human myeloid leukemias. *Vet Immunol Immunopathol*. 2010;135:243-256.
14. Horner BM, Cina RA, Wikiel KJ, et al. Predictors of organ allograft tolerance following hematopoietic cell transplantation. *Am J Transplant*. 2006;6:2894-2902.
15. Cho PS, Lo DP, Wikiel KJ, et al. Establishment of transplantable porcine tumor cell lines derived from MHC inbred miniature swine. *Blood*. 2007;110:3996-4004.
16. Sharabi Y, Sachs DH. Mixed chimerism and permanent specific transplantation tolerance induced by a nonlethal preparative regimen. *J Exp Med*. 1989;169:493-502.
17. Tomita Y, Sachs DH, Sykes M. Myelosuppressive conditioning is required to achieve engraftment of pluripotent stem cells contained in moderate doses of syngeneic bone marrow. *Blood*. 1994;83:939-948.
18. Majhail NS. Old and new cancers after hematopoietic-cell transplantation. *Hematology Am Soc Hematol Educ Program*. 2008;1:142-149.
19. Wamala I, Matar AJ, Farkash E, et al. Recombinant anti-monkey CD3 immunotoxin depletes peripheral lymph node T lymphocytes more effectively than rabbit anti-thymocyte globulin in naive baboons. *Transpl Immunol*. 2013;29:60-63.
20. Ghobrial IM, Habermann TM, Maurer MJ, et al. Prognostic analysis for survival in adult solid organ transplant recipients with post-transplantation lymphoproliferative disorders. *J Clin Oncol*. 2005;23:7574-7582.